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ABSTRACT:

PROBLEM TO BE SOLVED: To obtain the subject material usable for a food, a beverage and an agent for the oral cavity, capable of strongly preventing adhesion of glucan and microorganisms of decayed tooth to the surface of a tooth, comprising a hop bract as a raw material.

SOLUTION: This material is a polyphenol-like substance which is contained in a hop bract and is adsorbed on a gel type synthetic resin and/or a substance not passing through an ultrafilter having  $\geq 1,000$  fractional molecular weight when treated with the ultrafilter. A method for extracting a hop bract with water or an aqueous solution of an organic solvent immiscible with water, passing the extracted solution through a gel type synthetic adsorbent, cleaning the filtrate with the aqueous solution and eluting the fraction adsorbed on the adsorbent and/or a method for treating the extracted solution with the ultrafilter having  $\geq 1,000$  fractional molecular weight and removing the solvent from the residual solution may be cited as the method for obtaining the material.

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(54) ANTICARIOGENIC MATERIAL, ITS PRODUCTION AND USE

(57)Abstract:

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DETAILED DESCRIPTION

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## [Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the cariostatic material obtained from a hop bract, its manufacture approach, and its application.

[0002]

[Description of the Prior Art] Hop is the perennial plant of Moraceae and, generally is calling hop the cone (that in which the non-fertilized female flower matured). The lupulin parts (granulation of the yellow formed in the root of \*\* among cones) of this hop are the bitterness of hop, and the body of aroma, and are the important Biel raw materials together with yeast and malt in a brewery. Moreover, hop is accepted as a sedative or an anti-aphrodisiac with folk remedies. It is said that the bodies of various operations of these hop lupulin parts are bitter acid, such as alpha acid and beta acid, and inhibition (JP,06-25000,A) of a protease operation useful for prevention or the therapy of prevention of cariosity (JP,63-211219,A), an antibacterial action (JP,06-98738,A), and gum disease, the antioxidation operation (JP,04-202138,A, JP,06-025081,A, JP,06-312924,A), the superoxide elimination operation (JP,04-5237,A), etc. are already clarified as a technique using the alpha acid and beta acid.

[0003] On the other hand, a hop bract removes a lupulin part from a hop cone, it is not supposed at a brewery that it is useful, but in the case of a brewery, a hop bract is removed depending on the case and produced as a by-product. In that case, especially effective directions are not found out except that a hop bract is used as a fertilizer for soil amelioration, but development of directions with more high added value is desired. In addition, in Japanese Patent Application No. No. 173931 [ 07 to ] concerning application of these people, it is checking having an antioxidation operation about the polyphenol pharmaceutical preparation of hop, especially the hop bract origin. Moreover, in Japanese Patent Application No. No. 347151 [ 07 to ] concerning application of these people, it uses for the foaming malt beverage by using the extract of hop, especially the hop bract origin as a bubble stabilizing agent.

[0004] As a cariostatic material which uses natural materials other than hop as a raw material, and makes polyphenol an active principle, a green tea extract (JP,01-9922,A, JP,01-90124,A, JP,01-265010,A), an oolong tea extract (JP,03-284625,A), apple polyphenol (JP,07-285876,A), etc. are marketed.

[0005]

[Problem(s) to be Solved by the Invention] Although they are a disease it is supposed that was existed from B.C. 2,000 or before, since many [ with dental caries and the so-called cavity / when not being directly related to a life ], they have not resulted in the extermination till today. However, in the odontopathy survey of the Ministry of Health and Welfare, the people with a certain dental caries are supposed that about 85% of Japanese people are reached, and can say one [ so-called ] of the national illnesses.

[0006] It is thought that dental caries is a disease caused with bacteria. That is, from cane sugar, adhesiveness and insoluble glucan are generated, and with a fungus body, the so-called "cavity bacilli", such as S.mutans which exists in the oral cavity first, and S.sobrinus, adheres on the surface of a gear tooth, and forms a dental plaque. Subsequently, various bacilli, such as a cavity bacillus, breed in this dental plaque, pH on the front face of a gear tooth falls for the organic acid generated by the metabolic turnover of a bacillus, dental enamel begins to melt, a hole (deashing) is vacant, and it is supposed that dental caries is produced.

[0007] The approach using the alternative sugar which does not generate glucan, the approach using the inhibitor of the enzyme which generates glucan, etc. are already learned instead of the approach of strengthening dentine, using a fluorine as an approach of preventing dental caries, the approach using the antimicrobial to a cavity bacillus, and cane sugar. However, it has merits and demerits in respect of the effect which gives any approach to effectiveness, and cost and a nutrition, and even if it considers that the morbidity of a caries puts actually and is not decreasing, it is hard to

say that it is a perfect approach. Then, if the outstanding approach of preventing a caries temporarily is developed, it is greatly industrially meaningful.

[0008] Therefore, rather than the cariostatic material which makes an active principle the polyphenol of the natural origin by which current marketing is carried out, the purpose of this invention obtains a cariostatic material with still stronger activity from not a lupulin part but the hop bract of hop, and is in the point of using this for an eating-and-drinking article or the agent for the oral cavities.

[0009]

[Means for Solving the Problem] this invention persons found out that it was the matter with which the matter which was polyphenol Mr. matter contained in a hop bract as a result of examining the above-mentioned technical problem wholeheartedly, processed by gel type composition resin or ultrafiltration membrane, and was obtained checks powerfully adhesion of the glucan on the front face of a gear tooth and a cavity bacillus, i.e., it is the matter which can be used as a cariostatic material. Furthermore, it came to complete this invention by using this matter for an eating-and-drinking article or the agent for the oral cavities as a cariostatic material.

[0010] It is related with the cariostatic material which the 1st is the polyphenol Mr. matter contained in the hop bract of this invention, and is the matter which sticks to gel type composition resin. Namely, the 2nd It is the polyphenol Mr. matter which contains the 3rd in a hop bract about the cariostatic material which is the matter which does not penetrate the film by the polyphenol Mr. matter contained in a hop bract when a cut off molecular weight processes by 1,000 or more ultrafiltration membrane. It is related with the cariostatic material which is the matter which sticks to gel type composition resin, and is the matter which does not penetrate the film when a cut off molecular weight processes by 1,000 or more ultrafiltration membrane.

[0011] The cariostatic material in said 1st invention extracts a hop bract in the water solution of the organic solvent with which it mixes with water or water, and is characterized by eluting after washing the fraction which stuck to said adsorbent with the water solution of the organic solvent with which it mixes with water further, and being manufactured in the water solution of the organic solvent which mixes with the extract with water or water through a gel type composition adsorbent, (how to use a gel type composition adsorbent).

[0012] The water solution of the organic solvent with which it mixes with water or water extracts a hop bract, a cut off molecular weight processes the extract by 1,000 or more ultrafiltration membrane, and the cariostatic material in the 2nd invention is characterized by removing a solvent from the upper remaining liquid, and being manufactured (approach by ultrafiltration membrane). The cariostatic material in the 3rd invention is characterized by being obtained combining the approach of using said gel type composition adsorbent, and the approach by said ultrafiltration membrane. Moreover, this invention relates to the eating-and-drinking article containing the above-mentioned cariostatic material, and the agent for the oral cavities.

[0013]

[Embodiment of the Invention] From a hop cone, the hop bract used as the raw material of this invention removes a lupulin part, and is obtained, and, generally a hop bract is obtained by removing a lupulin part with sieving after grinding a hop cone. however, the \*\* which does not remove to a brewery the hop bract which is not useful in order to save the time and effort which sifts out and removes a hop bract in the latest brewery -- a hop cone -- it remains as it is -- it fabricates to a pellet type and is in the inclination used for a brewery as a hop pellet. Therefore, it is satisfactory at all also considering the hop cone and hop pellet which will not limit as a raw material of this invention especially if a hop bract is included, but contain a hop bract as a raw material.

[0014] The water solution of the organic solvent with which it mixes with water, such as water or alcohol not more than 50 v/v%, an acetone, and an acetonitrile, extracts the hop cone which contains the hop bract or hop bract which is a raw material first as a manufacturing method of a cariostatic material, a hop pellet, etc. As a suitable example, water or the water ethanol not more than ethanol 50 v/v% is mentioned. The rate of a raw material and an extracting solvent has 1:20 to 100 (weight ratio) desirable extent, and, as for an extract, it is desirable under 4-95 degrees C and stirring to be carried out a grade for 20 - 60 minutes. Although an extract is obtained by filtration, if there is need in that case, filtration assistant \*\*, such as a perlite, can also be used.

[0015] Thus, the obtained extract is processed by the gel type composition adsorbent and/or ultrafiltration membrane.

\*\* About the extract of the raw material obtained at the processing above-mentioned extract process by the gel type composition adsorbent, perform the elution process eluted in an adsorption fraction from a gel type composition adsorbent by the adsorption process which makes a cariostatic material stick to a gel type composition adsorbent, water or the ethanol water solution, the washing process that washes a gel type composition adsorbent with 1 - 10 v/v% of ethanol water solution preferably, the ethanol water solution beyond 30 v/v%, or ethanol, and obtain a cariostatic material.

[0016] An adsorption process is a process which it dips [ process ] in the column filled up with the gel type composition adsorbent, and makes a cariostatic material stick to an adsorbent, after cooling this extract solution to room temperature extent of 15-30 degrees C. If there is need in that case, in order to gather adsorption effectiveness, it is desirable to lower the organic solvent concentration of an extract to 10% or less beforehand with vacuum concentration etc. As the quality of the material of a gel type composition adsorbent, a hydrophilic vinyl polymer, hydroxypropyl-ized dextran, and styrene-divinylbenzene polymer etc. can be mentioned. As for dipping time amount, it is desirable to set up so that SV value may become between 0.5-100. In addition, SV value said here is a value defined by the following formulas.

[0017]  $SV \text{ value} = (\text{amount of dipping (L)}) / \{ (\text{the amount of resin (L)}) \times (\text{dipping time amount (h)}) \}$

A washing process is a process which washes the gel type composition adsorbent holding a cariostatic material, and becomes possible [ raising whenever / purification / of a cariostatic material / more except for a contaminant according to this process ]. As a solvent used for washing, water thru/or 1 - 10 v/v% of ethanol water solution are suitable, and it is desirable to dip and wash the amount of solvents which is about 1 to 10 times of the amount of resin.

[0018] An elution process is a process which carries out desorption elution of the cariostatic material from the gel type composition adsorbent holding a cariostatic material, as a solvent used for elution, water alcohol, a water acetone, a water acetonitrile, etc. can be used, and the ethanol water solution or ethanol beyond 30 v/v% is especially mentioned as a suitable example. The amount of dipping of an elution solvent has about 1 to 6 desirable times of the amount of resin.

[0019] Vacuum concentration of the obtained eluate can be carried out, and a cariostatic material can be obtained as powder except for a solvent by the usual approaches, such as freeze drying and spray dry. Moreover, solvents, such as alcohol, an acetone, and an acetonitrile, can also be collected and reused in the case of vacuum concentration. Thus, the obtained cariostatic material is the powder of the odorless flesh color which presented bitterness faintly, brown, or light yellow, and when the anti-caries activity body sticks to gel type composition resin and a cut off molecular weight processes it by 1,000 or more ultrafiltration membrane, it is polyphenol Mr. matter which does not penetrate the film.

[0020] In addition, yield is 0.5 - 15.0 w/w% in 0.5 - 20.0 w/w% and hop cone weight conversion at hop bract weight conversion. After the alcoholic water solution beyond 80 v/v%, an about [ 0.05N ] sodium-hydroxide water solution, etc. wash the used gel type composition adsorbent, it can be used repeatedly.

[0021] \*\* A cut off molecular weight processes the extract of the hop bract obtained at the processing above-mentioned extract process by ultrafiltration membrane by 1,000 or more ultrafiltration membrane. If there is need in that case, vacuum concentration of the extract can be carried out and organic solvent concentration can also be lowered. The collected organic solvent is also reusable. As a membranous material, if a cellulose, cellulose acetate, Pori Sall John, polypropylene, polyester, polyether sulphone, PVDF, etc. are usually used as the quality of the material of ultrafiltration membrane, it can use without a limit especially. Moreover, with [ a cut off molecular weight ] 1,000 [ or more ], it can be used especially satisfactory, but if the film with a not much large cut off molecular weight is used, since the time amount which yield falls extremely, and processing takes when a cut off molecular weight is small will become long, the ultrafiltration membrane of cuts off molecular weight 10,000-20,000 is suitable. Moreover, although processing is based also on the class of extracting solvent, or the rate of an extracting solvent, hop, or a hop bract, it is desirable to carry out until the amount of the upper remaining liquid becomes about 1 at the time of processing initiation / ten to 1/100 about. Although the pressure in that case is based also on ultrafiltration membrane or a filter, it is desirable that it is about 0.1-10.0kg/cm<sup>2</sup>. Moreover, if there is need, the upper remaining liquid processed once can be again thinned with suitable solvents, such as water, it can rework similarly, and whenever [ purification ] can also be raised.

[0022] Except for the solvent of the obtained upper remaining liquid, a cariostatic material can be obtained as powder by the usual approaches, such as concentration, freeze drying, and spray dry. Moreover, solvents, such as alcohol, an acetone, and an acetonitrile, can also be collected and reused in the case of vacuum concentration. Thus, the obtained cariostatic material is the powder of the odorless flesh color which presented bitterness faintly, brown, or light yellow, and when the anti-caries activity body sticks to gel type composition resin and a cut off molecular weight processes it by 1,000 or more ultrafiltration membrane, it is polyphenol Mr. matter which does not penetrate the film.

[0023] In addition, yield is 0.5 - 15.0 w/w% in 0.5 - 20.0 w/w% and hop cone weight conversion at hop bract weight conversion. Since the activity bodies of the cariostatic material obtained by the processing by the above-mentioned \*\* gel type composition adsorbent or processing by \*\* ultrafiltration membrane are the same polyphenol, they can dissolve the cariostatic material obtained by processing by the gel type composition adsorbent in suitable solvents, such as an alcoholic water solution, and can also raise whenever [ purification / of the polyphenol which is an activity body further ] by processing by ultrafiltration membrane. Moreover, the reverse is also possible and a cariostatic material

also with the approach independent of \*\* or \*\* useful enough, of course can be obtained.

[0024] the obtained cariostatic material -- eating-and-drinking articles, such as confectionary, food, and a drink, -- Kandy, chocolate, a caramel, chewing gum, etc. can be especially used for the comparatively long eating-and-drinking article of the oral cavity residence time preferably. Moreover, it can also add and use for agents for the oral cavities, such as mouth washing and dentifrices. Although a cariostatic material may be added with powder in case a cariostatic material is added to such eating-and-drinking articles and the agent for the oral cavities, it is desirable to use a cariostatic material as the solution or the alcoholic solution of 1 - 2% of water solution or an alcoholic water solution, and for the last concentration to add to an eating-and-drinking article or the agent for the oral cavities, preferably, so that 1-500 ppm may turn into 10-100 ppm preferably.

[0025] In addition, as mentioned above, using the extract of hop as a cariostatic material is already indicated by JP,62-211219,A. said -- an official report -- depending -- if -- "S. -- mutans -- a bacillus -- growth -- effective -- stopping -- and -- insolubility -- glucan -- generation -- control -- being effective -- hop -- an extract -- isomerization -- an object -- and -- the -- a metal -- a salt -- a caries -- preventive -- \*\*\*\*\* -- providing -- " -- things -- it can do. However, this invention article can prevent a caries, without exerting change on the condition of the natural bacterial flora in the oral cavity, since it does not have fungistatic [ over an S.mutans bacillus / antibacterial and fungistatic ] unlike an antibiotic etc. Moreover, alpha acids (humulone) and beta acids (lupulone) which are mentioned to JP,62-211219,A as an example of a hop extract are the principal component of the lupulin part of hop, and are a component which is not contained in the hop bract part which is the raw material of this invention article. Furthermore, in JP,62-211219,A, although [ the loadings of caries preventive ] "A caries preventive effect is preferably discovered effectively in 0.1 - 10 % of the weight 0.01 to 20% of the weight", this invention article shows effective effectiveness with the loadings of 1/10 - 1/the 100 (since it is equivalent to 1000 ppm 0.1% of the weight).

[0026]

[Example] Hereafter, although an example is shown, this invention is not limited to this.

Example 1 (preparation of the cariostatic material from the hop cone by the gel type composition adsorbent)

The mortar ground 20g of hop cones, and 2l. water extracted 95 degrees C for 40 minutes under stirring. After filtration, it cooled radiationally, and it dipped, having applied it to the column filled up with 80ml (Toyo Perl HW 40 by TOSOH CORP.) of hydrophilic vinyl polymer resin for 2 hours (SV=12.5), and, subsequently the ethanol water solution washed 5 400ml%. Furthermore, 400ml of ethanol water solutions was dipped in this column 80%, and these eluates were collected, and it freeze-dried and obtained as powder of the light yellow which presented bitterness faintly [ 800mg of cariostatic materials / no odor ]. The yield from hop was 4%.

[0027] Example 2 (preparation of the cariostatic material from the hop bract by the gel type composition adsorbent)

The ethanol water solution extracted 20g of 80 degrees C of hop bracts for 40 minutes under stirring 50 600ml%.

Vacuum concentration was carried out after filtration until the volume was set to 300ml, and it dipped, having concentration covered [ the ] it over the column filled up with 80ml (Mitsubishi Chemical SEPABIZU 825) of styrene-divinylbenzene resin for 1 hour (SV=3.75), and, subsequently 400ml water washed. Furthermore, 400ml of ethanol water solutions was dipped in this column 80%, and these eluates were collected, and it freeze-dried and obtained as powder of the light yellow which presented bitterness faintly [ 1.6g of cariostatic materials / no odor ]. The yield from a hop bract was 8%.

[0028] Example 3 (preparation of the cariostatic material from the hop cone by ultrafiltration membrane)

The mortar ground 20g of hop cones, and 2l. water extracted 95 degrees C for 40 minutes under stirring. After filtration, it cooled radiationally, and by the ultrafiltration membrane (Amicon XM50) of 50,000, under 1.0kg/cm<sup>2</sup> and a room temperature, the cut off molecular weight processed the extract until it was set to 20ml. Reduced pressure hardening by drying of the obtained upper remaining liquid was carried out, and it obtained as powder of the light yellow which presented bitterness faintly [ 200mg of cariostatic materials / no odor ]. The yield from hop was 1%.

[0029] Example 4 (preparation of the cariostatic material from the hop bract by ultrafiltration membrane)

The ethanol water solution extracted 20g of 80 degrees C of hop bracts for 40 minutes under stirring 50 600ml%. After filtration, under 3.0kg/cm<sup>2</sup> and a room temperature, the cut off molecular weight processed the extract by the ultrafiltration membrane (Amicon YM10) of 10,000 until it was set to 60ml. The obtained upper remaining liquid was freeze-dried and it obtained as powder of the light yellow which presented bitterness faintly [ 0.8g of cariostatic materials / no odor ]. The yield from a hop bract was 4%.

[0030] Example 5 (preparation of the cariostatic material from the example 2 by ultrafiltration membrane)

It dissolved in the ethanol water solution 10 500ml%, and by the ultrafiltration membrane (Amicon YM10) of 10,000, under 1.0kg/cm<sup>2</sup> and a room temperature, the cut off molecular weight processed 0.8g of cariostatic materials obtained in the example 2 until it was set to 20ml. The obtained upper remaining liquid was freeze-dried and it obtained as



powder of the light yellow which presented bitterness faintly [ 0.4g of cariostatic materials / no odor ]. When HPLC analysis of this powder was carried out, and the catechin quantum was performed by the approach which became characteristic chromatogram as shown in drawing 1 , and was indicated by the food official method of analysis, it converted into the catechin content and 40.6% of value was acquired.

(HPLC conditions) equipment: -- Shimazu LC-10A system and column:ODS-80TM (TOSOH --) 4.6mm I.D.x25cm and mobile phase:(A liquid: B liquid) = (100:0) from -- \*\* (50:50) up to -- the straight-line gradient for 30 minutes -- A liquid: 5% acetonitrile (0.1% HCl content), B liquid:acetonitrile, rate-of-flow:1.0 ml/min, and sample injection rate:20microg, multi-wavelength detection by detection:200-300nm.

[0031] Example 6 (presumption of the molecular weight of the example 5 by the gel filtration)

The molecular weight of the cariostatic material which performed the gel filtration on the following HPLC conditions, and was obtained in the example 5 was presumed. As compared with a calibration curve, molecular weight is about 36,000-40,000 about the holding time.

Equipment: (HPLC conditions) Shimazu LC-8A system and column:TOSOH TSK-GEL G4000PWXL, G5000PWXL, mobile phase: 0.1 M Tris-HCl Buffer solution (pH10.0): 2-propanol =1:1, detection:UV220nm, marker:LMW GEL FILTRATION KIT (Pharmacia Biotech), rate-of-flow:0.3 ml/min

Example 7 (dentifrices)

Dibasic calcium phosphate 42.0 glycerols 18.0 carrageenan 0.7 sodium lauryl sulfate 1.2 saccharin sodium 0.09 butyl parahydroxybenzoate Material obtained in the 0.005 examples 1 0.005 scents Charge 1.0 water 37.0 \*\* Total According to the conventional method, it considered as dentifrices using each component of each weight section of the 100.0 above. In addition, the dentifrices which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 were obtained similarly.

[0032] Example 8 (mouthwash)

A glycerol 7.0 sorbitols 5.0 ethyl alcohol 15.0 sodium lauryl sulfate 0.8 saccharin sodium 0.1 1-menthol 0.05 scents Charge Material obtained in the 0.045 examples 1 0.005 water 72.0 \*\* Total According to the conventional method, it considered as the mouthwash using each component of each weight section of the 100.0 above. In addition, the mouth wash which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 was obtained similarly.

[0033] Example 9 (trochiscus)

Gum arabic 6.0 magnesium stearates 3.0 grape sugars 73.0 milk Sugar The 2nd potassium of 17.6 phosphoric acids The 1st potassium of 0.2 phosphoric acids 0.1 scents Charge Material obtained in the 0.095 examples 1 0.005 \*\* Total According to the conventional method, it considered as the trochiscus using each component of each weight section of the 100.0 above. In addition, the trochiscus which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 was obtained similarly.

[0034] Example 10 (candy)

Cane sugar 20.0 starch syrups (75% solid content) 70.0 water 9.5 coloring agents 0.45 scents Charge Material obtained in the 0.045 examples 1 0.005 \*\* Total According to the conventional method, it considered as the candy using each component of each weight section of the 100.0 above. In addition, the candy which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 was obtained similarly.

[0035] Example 11 (chewing gum)

Gum base 20.0 calcium carbonates 2.0 milk Sugar 77.0 stevioside Material obtained in the 0.095 examples 1 0.005 scents Charge 0.9 \*\* Total According to the conventional method, it considered as chewing gum using each component of each weight section of the 100.0 above. In addition, instead of the example 1, the chewing gum which added examples 2, 3, 4, and 5, respectively was obtained similarly.

[0036] Example 12 (juice)

Concentration mandarin orange fruit juice 15.0 \*\* Sugar 5.0 citric acids 0.2 scents Charge 0.1 colors Base 0.15 sodium ascorbate Material obtained in the 0.048 examples 1 0.002 water 79.5 \*\* Total According to the conventional method, it considered as juice using each component of each weight section of the 100.0 above. In addition, the juice which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 was obtained similarly.

[0037] Example 13 (Cookie)

Weak flour 32.0 \*\* Egg 16.0 butter 16.0 sand Sugar 25.0 water 10.8 baking powder Material obtained in the 0.198 examples 1 0.002 \*\* Total According to the conventional method, it considered as Cookie using each component of each weight section of the 100.0 above. In addition, the Cookie which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 was obtained similarly.



## [0038] Example 14 (caramel)

Granulated sugar 31.0 starch syrups (75% solid content) 20.0 powder Milk 40.0 hardened oil 5.0 meals Salt 0.6 scents Charge Material obtained in the 0.025 examples 1 0.005 water 3.37 \*\* Total According to the conventional method, it considered as the caramel using each component of each weight section of the 100.0 above. In addition, the caramel which added the material obtained in the examples 2, 3, 4, and 5, respectively instead of the material obtained in the example 1 was obtained similarly.

## [0039] Example 1 of a comparison (preparation of a hop pellet extract)

The mortar ground hop pellet (from DOITSUHARATAU) 20g, and 80 degrees C was extracted for 40 minutes by 600ml 50% ethanol. After the extract, it filtered and the at-long-intervals alignment of the extract was carried out for 3,000rpmx 15 minutes. The supernatant liquid was freeze-dried and 6.3g of hop pellet extracts was obtained as light green powder.

## [0040] Example 2 of a comparison (purification of alpha acid and beta acid)

The peak of alpha acid (humulone) separated from the mixed elegance of the hop bitterness component purchased from the Switzerland brewing examination site on the following HPLC conditions and beta acid (lupulone) was isolated preparatively and refined. The yield from the mixed elegance of a hop bitterness component was 8% of alpha acids, and 5.5% of beta acids.

Equipment: (HPLC conditions) Shimazu LC-8A system and column:Shim-pack PREP-ODS (20mmI.D.x25cm), a mobile phase:80% acetonitrile water solution, rate-of-flow:10 ml/min.

## [0041] Example 15 (caries [ anti-] activity: adhesion depressor effect of a cavity bacillus)

The oolong tea origin material of marketing from which the cariostatic material obtained in the examples 1-5 and their food material with cariostatic are got (Sun Wu Ron: Suntory), A green tea origin material (SANFURABON HV: TAIYO KAGAKU, polyphenon 60:Mitsui agriculture and forestry), An apple origin material (Apple Computer phenon: NIKKA WHISKY DISTILLING) and the epigallocatechin gallate which is a typical natural product with cariostatic (Kurita Water Industries), It experimented by referring to the approach (S.Hamada & M.Torii, Infect.Immun., 20, 592-596) of Hamada and others about the hop pellet extract obtained in the example 1 of a comparison, alpha acid (humulone) obtained in the example 2 of a comparison, and beta acid (lupulone). S.mutans (Institute of Physical and Chemical Research preservation strain 5175 MT8148) and S.sobrinus (ATCC preservation strain 33478) which are strain typical as a cavity bacillus were used. 2ml of 0.5mg [ml] cavity bacilli, 0.2ml (S. in the case of sobrinus 1%) of 10% cane sugars, and 0.2ml (all solution of 50mM phosphate buffer solution (pH6.8)) of sample offering matter of each concentration were mixed, and 37 degrees C incubated for 18 hours among the test tube which made 30 degrees incline. After removing mixed liquor calmly, the test tube was quietly washed with the 3ml phosphate buffer solution. The cell mass adhering to the inside of mixed liquor and a washings and a test tube wall was measured with the turbidity in 550nm, respectively, and the sample offering matter concentration which reduces by half the cell mass which remains in a test tube wall was expressed as an index (IC50:mug/ml) of anti-caries activity. The result is shown in Table 1. The cariostatic material which the material obtained in the example 1 of a comparison, alpha acid, and beta acid did not show activity, and was obtained in the examples 1-5 compared with IC50 of epigallocatechin gallate and a commercial cariostatic material having been 20-200microg/ml showed the very strong anti-caries activity of about 2-15microg/ml in IC50.

## [0042]

Table 1 Adhesion control activity of cavity bacillus (IC50:mug/ml)

A sample S. mutans S. The material obtained in the sobrinus example 1 3.2 The material obtained in the 11.1 examples 2 2.6 The material obtained in the 10.6 examples 3 2.5 The material obtained in the 14.1 examples 4 10.4 The material obtained in the 13.9 examples 5 1.5 A 8.5 oolong-tea origin material 20.2 The 118.5 green-tea origin material 1 26.6 170.6 green-tea origin material 2 A 42.0 208.5 apple origin material 64.3 148.3 epigallocatechin gallate 22.4 Material obtained in the example 1 of 69.7 comparisons >200 >400alpha acid (humulone) >200 >400beta acid (lupulone) > 200 > 400 [0043] Example 16 (growth control activity of a cavity bacillus)

It investigated about the effect which it has on growth of S.mutans (Institute of Physical and Chemical Research preservation strain 5175 MT8148) which is the typical strain of a cavity bacillus about the streptomycin (streptomycin sulfate: Meiji Seika Kaisha, Ltd.) which is the cariostatic material obtained in the examples 1-5, the hop pellet extract obtained in the example 1 of a comparison, alpha acid (humulone) obtained in the example 2 of a comparison, beta acid (lupulone), and typical antimicrobial. Namely, 1% cane-sugar addition Todd Hewitte Broth 0.1% (1000 ppm) of each sample offering matter was added to the culture medium, and the S.mutans bacillus was \*\*\*\*(ed) by the platinum loop and cultivated 37 degrees C for 20 hours. Water was used as contrast. If a bacillus increases, an organic acid will produce by the increase of the turbidity of a culture medium, and the metabolic turnover of a bacillus, and pH of a

culture medium will fall. Turbidity before and behind culture (OD660nm) And change of pH was measured and it considered as the index of growth of a bacillus. The result is shown in Table 2. Although the material obtained in the example 1 of a comparison, alpha acid, and beta acid controlled growth of a bacillus completely, the material obtained in the examples 1-5 did not affect growth of a cavity bacillus.

[0044]

Table 2 Cavity bacillus (*S. mutans*) Effect which it has on growth Sample Turbidity rise value Before [ pH ] culture After [ pH ] culture Contrast(water) 0.302 7.67 4.28 Material obtained in the example 1 0.288 7.73 4.55 Material obtained in the example 2 0.305 7.68 4.37 Material obtained in the example 3 0.297 7.71 4.26 Material obtained in the example 4 0.300 7.66 4.58 Material obtained in the example 5 0.292 7.69 4.65 Material obtained in the example 1 of a comparison 0.001 7.68 7.69 alpha acid (humulone) 0.002 7.66 7.62 beta acid (lupulone) 0.000 7.71 7.71 Streptomycin 0.001 7.68 7.66 [0045] The minimum effective inhibition concentration to *S. mutans* (Institute of Physical and Chemical Research preservation strain 5175 MT8148) which is strain with a cavity bacillus typical about the streptomycin (streptomycin sulfate: Meiji Seika Kaisha, Ltd.) which is the cariostatic material obtained in the example 17 (antimicrobial activity by Media Interface Connector measurement) examples 1-5, the hop pellet extract obtained in the example 1 of a comparison, alpha acid (humulone) obtained in the example 2 of a comparison, beta acid (lupulone), and typical antimicrobial (Media Interface Connector) It measured. Culture medium Todd Hewitte Broth Culture was performed for two days at 37 degrees C using the culture medium. The result is shown in Table 3. The material obtained in the examples 1-5 did not control growth of a cavity bacillus in the amount to the material obtained in the example 1 of a comparison, alpha acid, and beta acid having shown 10-100microg [ /ml ] Media Interface Connector the 100 to 1000 times.

Table 3 Antimicrobial activity to cavity bacillus (mug/ml)

Sample Material obtained in the Media Interface Connector example 1 Material obtained in the >30 example 2 Material obtained in the >30 example 3 Material obtained in the >30 example 4 Material obtained in the >30 example 5 Material obtained in the example 1 of >30 comparison 5.0 alpha acid (humulone) 1.0 beta acid (lupulone) 1.0 streptomycin 0.5

[0046] Example 18 (inhibitory action to the insoluble glucan generation enzyme of a cavity bacillus)

The cariostatic material, the commercial oolong tea origin material (Sun Wu Ron: Suntory) which were obtained in the examples 2 and 5, and about the epigallocatechin gallate (Kurita Water Industries) which is a typical natural product with cariostatic the approach of Nakahara and others (it Nakahara(s) K. --) S.Kawabata, H.Ono, K.Ogura, T.Tanaka, T.Ooshima, and S.Hamada, Appl.Environ.Microbiol., 59, and 968-973 (1993) It was made reference and the inhibitory action to the insoluble glucan generation enzyme of a cavity bacillus was considered. *S. mutans* (Institute of Physical and Chemical Research preservation strain 5175 MT8148) and *S. sobrinus* (ATCC preservation strain 33478) which are a typical cavity bacillus were cultivated, respectively, and centrifugal processing separated the fungus body and the culture supernatant. *S. About mutans*, the fungus body was processed with 8M urea, processing liquid was dialyzed, and the rough liquid of an insoluble glucan generation enzyme was obtained. *S. About sobrinus*, the culture supernatant was salted out with the ammonium sulfate 50%, precipitate was dialyzed, and the rough liquid of an insoluble glucan generation enzyme was obtained. These enzyme rough liquid, a test sample, and shoe cloth (final concentration 50mM) were used as the mixed solution of the 100mM potassium phosphate buffer solution (pH6.5) of 750micro of whole quantity 1, and 25 degrees C incubated for 16 hours. At-long-intervals alignment processing of the mixed liquor is carried out 15,000 rpm after incubation for 10 minutes, and Mr. postprecipitation except supernatant liquid was washed 3 times with the 100mM potassium phosphate buffer solution (pH6.5) and 1:1 mixed liquor of ethanol. The precipitate was dissolved in the 1-N sodium-hydroxide water solution, and the quantum of sugar was performed by the phenol sulfuric-acid method. S. the rate of inhibition of each material to mutans and *S. sobrinus* -- drawing 2 (a) and (b) It is shown. Consequently, the cariostatic material obtained in the example 2 and the example 5 showed enzyme inhibition activity stronger than an oolong tea origin material and epigallocatechin gallate. (<> in drawing: Express an example 5, the \*\*:example 2, \*\*:epigallocatechin gallate, and x:oolong tea origin material, respectively.)

It became clear not to have fungistatic [ over a cavity bacillus / antibacterial and fungistatic ], and to control adhesion in the tooth flank of a cavity bacillus powerfully as a result of examples 14-16. Furthermore from an example 17, it is thought that the effectiveness is the inhibitory action to the generation enzyme of insoluble glucan.

[0047]

[Effect of the Invention] According to this invention, the cariostatic material with very strong activity was able to be obtained rather than the commercial cariostatic material by using the hop cone containing a hop bract and a hop bract etc. as a raw material. Furthermore, as a cariostatic material of the vegetable origin, it was able to use easily as an eating-and-drinking article or an agent for the oral cavities.

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[Translation done.]